Colony-stimulating factors and antibiotics—a new prospect in treating infectious diseases?

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Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are glycoproteins which have substantial effects on the proliferation and differentiation of neutrophils, the latter also influencing macrophages. They are therapeutically used in cases where increasing the neutrophil count is essential, as in neutropenic, immunocompromised or cancer patients. However, an additional effect of CSF on the activation of mature neutrophils has been demonstrated in experimental models [1,2]. The predominant function of neutrophils in cellular defense suggests a key position of CSF, which is underlined by its increase during infection. This aroused interest in using CSF for specifically improving the immune response during infections in non-neutropenic patients without malignancies. Unlike the regular indications, where CSF acts prophylactically, this indication is focused on the immediate cell-activating therapeutic effect after onset of infection which might act additionally or even in synergy with antibiotics. G-CSF and GM-CSF have therefore been investigated in numerous studies examining the immune modulating effect and possible synergy with antibiotics as well as side effects, in vitro, in animal infection models, and with activated polymorphonuclear leukocytes (PMNLs) from CSF-treated patients or volunteers. There are also some reports on the therapeutic use of CSF in patients with severe infections.

The in vitro synergistic effect resulting from combining G-CSF and antibiotics was demonstrated by killing of *Escherichia coli* in the presence of G-CSF and ceftazidime or ofloxacin [3,4] . Animal models showed the positive effect of G-CSF on different types of infection. Of special interest are those with an underlying immunologic dysfunction other than neutropenia, such as infection after trauma, burn, intoxication, splenectomy or neonatal sepsis. Neonatal PMNLs are deficient in chemotaxis and show a reduced oxidative response in times of stress. As neonatal sepsis is usually characterized by neutropenia, efficacy of G-CSF in therapeutic treatment may be due to both the granulopoietic and the stimulating effects. In a model of experimental group B streptococcus (GBS) sepsis in newborn rats, G-CSF and ampicillin (150 mg/kg per day) or gentamicin (6.5 mg/kg per day) had a significant synergistic effect on survival. Treatment with G-CSF alone yielded a 9% survival rate, single antibiotic treatment 28% and combined treatment 91%, versus 4% in the control group ($p \leq 0.001$). G-CSF pulse prophylaxis (5 μg/kg) 6 h before GBS inoculation combined with antibiotics increased survival to 70% versus 10% after single antibiotic treatment ($p \leq 0.01$) [5]. In a follow-up, 7-day prophylaxis versus pulse treatment 6 or 18 h after GBS inoculation was compared. Prophylaxis (5 μg/kg per day) significantly increased peripheral neutrophilia and the bone marrow neutrophil storage pool ($p \leq 0.001$). Its synergy with antibiotic treatment was shown by 48-h/72-h survival rates of 100% each with combined treatment, and 90%/50% with antibiotics alone, 40%/0% with G-CSF alone, versus 0% each in the controls. Pulse treatment, however, yielded neither synergy with antibiotics nor improved survival, and did not prevent neutropenia [6].

Trauma and burn patients with severe infections might possibly benefit from G-CSF treatment. In a mouse hemorrhage and trauma model, administration of G-CSF starting 2 or 4 days prior to and continuing for 7 days after induction of a *Pseudomonas aeruginosa* pneumonia increased survival to 38% versus 8% in controls. Significance could be achieved with a dose of 100 μg/kg per day, but not with 50 μg/kg per day [7]. Burned mice with *Pseudomonas aeruginosa* wound infection showed a significant improvement after gentamicin (once 6 mg/kg IP immediately after burn) and G-CSF treatment (200 ng/day, 7 days) alone or in combination, compared to controls ($p < 0.001$ for all groups). Combined treatment yielded higher survival rates than single gentamicin ($p = 0.007$) or single G-CSF administration ($p = 0.054$) [8].

In the immunocompetent host, management of systemic infections may also be difficult because often only a few effective antibiotics are available, which makes a single or a synergistic CSF treatment an important option. G-CSF treatment provided significant protection in a murine model of lethal fecal peritonitis. However, this effect was only obtained with 2 days' prophylaxis (100 μg/kg per day), and not with an immediate pretreatment [9]. In contrast to this, in a rat model of cecal ligation and puncture (CLP) a significant decrease in mortality was noted when G-CSF (15 μg IP) was administered once after onset of sepsis ($p < 0.001$) [10]. This was verified in a later study of a similar model, where G-CSF treatment at 20 μg/kg per day starting 4 h after sepsis induction

significantly reduced the mortality rate from 96% to 42% ($p<0.05$). This protection could not be significantly improved, either by increasing the dose or by an additional prophylactic application [11].

Combination of G-CSF (different doses) and gentamicin (1×15 mg/kg) was investigated in a murine CLP model. Single G-CSF treatment significantly increased survival, with dose- and time-dependent efficacy. Combined therapy yielded significantly improved survival compared to non-treated animals ($p=0.001$) and to single G-CSF treatment ($p=0.0475$) but not to single gentamicin treatment [12]. *Escherichia coli* peritonitis in rats had a significantly increased survival rate (78% versus 38%) when animals were treated with ceftriaxone (14.3 mg/kg) combined with a 2-day G-CSF pretreatment (100 μg/kg per day) [13]. In experimental disseminated candidosis in mice, G-CSF had no direct benefit, but combined with fluconazole could extend the survival beyond that for fluconazole alone [14]. Combined therapy with penicillin G and G-CSF gave a higher survival rate than penicillin G alone in a rabbit *Pasteurella multocida* pneumonia and sepsis model. Improvement was significant only in the group where sepsis was complicated by leukopenia. However, this effect mainly occurred prior to increase in neutrophil count, suggesting functional activation rather than neutrophil number or modulation of cytokine release being the cause [15]. Administration of G-CSF in catheter-related endocarditis seems to be less efficacious, as shown in a rabbit model of *Staphylococcus aureus* endocarditis, where G-CSF (25 μg/kg per day for 3 days) alone or combined with ceftriaxone (50mg/kg per day for 3 days) did not lead to an improved outcome [16]. In a rabbit *Pseudomonas aeruginosa* endocarditis model, G-CSF (100 μg/kg per day) given 3 days after bacterial challenge neither had an antibacterial effect nor increased the efficacy of ciprofloxacin (80 mg/kg per day). Only treatment starting 30 min prior to challenge yielded a significant but transient antimicrobial effect [17].

Up to now, there have been few reports published on treatment of infections in non-neutropenic patients with CSF. Liles et al examined the antifungal activity of PMNLs from three healthy normal human volunteers before and after G-CSF administration. The killing rate did not change significantly for *Candida albicans*, but increased four-fold for *Aspergillus fumigatus* and 15-fold for *Rhizopus arrhizus* ($p<0.05$). Absolute neutrophil count increased about six-fold after five G-CSF doses (300 mg/day SC). Enhancement of respiratory burst activity in response to extracts of hyphae and conidia was generally slower in onset but sustained in comparison to the rapid but brief respiratory burst after in vitro G-CSF pretreatment of control PMNLs [1]. In a prospective, controlled, randomized study, Gillan et al examined 42 newborn infants with presumed bacterial sepsis within the first 3 days of life. G-CSF was administered at 1, 5 and 10 μg/kg per day or 5 and 10 μg/kg per 12 h. Doses of 5 or 10 μg/kg induced a significant increase in absolute neutrophil count (ANC), with a maximum of 397% or 621% after 72 h, and a dose-dependent two- to three-fold increase in the neutrophil storage pool (NSP) was observed. A significant increase in neutrophil C3bi expression suggested a possible enhancement of PMNL adherence or aggregation leading to improved function and increased toxicity [18]. Nakazawa et al reported on a 2-month-old boy with a large multilocular *Pasteurella multocida* brain abscess. Intravenous injection of high doses of antibiotics and antibiotic irrigation of the abscess cavity could not prevent exacerbation by absent ANC increase. After additional rhG-CSF application (125 μg SC), neutrophil count was raised, and improved chemotaxis and superoxide anion production of the neutrophils was observed, resulting in prompt clinical and laboratory improvements [19]. Mueller-Werdan et al described the treatment of a patient with septic granulomatosis developing a severe sepsis with initial hemodynamic instability. As the infection seemed to be resistant to every antibiotic therapy tried, interferon was contraindicated for intolerance, and leukocyte transfusions were not available, G-CSF treatment (300 μg/day) was started, resulting in a dramatic clinical amelioration with temperature decrease and remission of disseminated intravascular coagulation (DIC). Antibiotics were stopped at the fifth day of G-CSF treatment. Three days later the patient was afebrile and showed no more symptoms of infection. Leukocyte analysis revealed an increased ANC but unchanged oxidative function [20]. Nelson et al investigated the use of G-CSF in the treatment of hospitalized patients with community-acquired pneumonia in a large multicenter double-blind trial. They enrolled 380 patients treated with G-CSF (300 μg/day SC for 10 days) and 376 placebo-treated controls. G-CSF elevated ANCs three-fold but did not lead to significant differences in mortality rate, time to resolution of morbidity and length of hospital stay. However, significant differences were noted on the intention-to-treat analysis. The comparison between treated versus control patients revealed adult respiratory distress syndrome in 4 versus 14 patients ($p=0.017$), DIC in 0 versus 7 patients ($p=0.007$) and empyema in 1 versus 7 patients ($p=0.037$). Median time to resolution in the chest radiograph was 29 days in treated patients versus 42 days in controls ($p=0.001$). Complete resolution in the chest radiograph by day 28

was found in 58% of treated versus 42% of control patients (p=0.005) [21].

In contrast to G-CSF, experimental models investigating the effect of GM-CSF did not reveal consistent results. In a CLP model, rats received a single dose of 20 μg GM-CSF 3 h after onset of infection. There was no improvement of survival rates after 48 h and even earlier deaths than in the control group were observed. Neutrophil counts in the peritoneal lavage fluid were lower in the GM-CSF-treated group, indicating inhibition of neutrophil migration [22]. In the above mentioned model of fecal peritonitis, G-CSF treatment yielded a significant protection whereas GM-CSF (50/100 μg/kg immediate or 2 days' prophylaxis) failed to protect animals, the mortality being 100%. It could not raise leukocyte counts and significantly increased levels of circulating TNF-α [9]. In a murine model of septic shock, G-CSF (50 g/kg) protected galactosamine-sensitized mice from lipopolysaccharide (LPS)-induced hepatitis and decreased LPS-induced serum TNF activity. In contrast, pretreatment with GM-CSF (50 μg/kg) resulted in significantly higher TNF activity and increased mortality [23].

However, in newborn rats with group B streptococcal sepsis combined, GM-CSF (1×0.05 μg/kg) and penicillin (3×100 mg/kg) treatment significantly decreased the mortality rate in comparison to penicillin alone (39% versus 76%) [24]. There are also some studies of wound and burn infections that have reported a positive effect of GM-CSF. In a model of *Pseudomonas aeruginosa*-infected burn injury, mice treated with GM-CSF (20 ng/day) had a significantly improved survival rate [25]. A significant increase in wound healing after GM-CSF treatment was found in a rat model of acute and chronic wound healing. Wound bacterial counts decreased in the treated group, with significance only at day 7 [26]. In a murine model of *Klebsiella pneumoniae* suture infection, the combination of GM-CSF, TNF-α, the immune adjuvant muramyl dipeptide and cefoxitin or ampicillin–sulbactam was investigated. After GM-CSF treatment, the survival rate was worse (2%) than in control animals (18%). Combination of immunomodulators yielded modest increases (50%). Combination of all immunostimulating substances with one antibiotic yielded significant increases in survival rate (84% cefoxitin, 90% ampicillin–sulbactam) [27].

Positive effects of GM-CSF treatment were shown in a report of a 42-year-old woman with underlying insulin dependent diabetes mellitus and chronic mucocutaneous candidosis resistant to conventional antifungals. Neutrophils showed reduced chemotaxis to bacterial factor and zymosyme. Treatment with GM-CSF 7 μg/kg per day for 2 weeks and on alternate days thereafter cleared all fungal lesions completely within 1 week and yielded a substantial improvement in neutrophil chemotaxis, an increased monocyte count and increased interleukin-1 secretion [28].

We conclude from this literature review that G-CSF and to a lesser extent GM-CSF seem to be efficacious for prophylaxis and as supplementary treatment of various infectious diseases. However, further clinical studies must be done in order to precisely define the indications and also to reveal potential side effects.

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